



NTP

National Toxicology Program

Toxicology and Carcinogenesis Studies of 4-Methylimidazole in F344 Rats and B6C3F₁ Mice

Po C. Chan, Ph.D.

National Toxicology Program

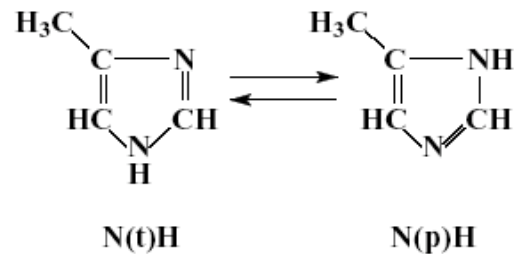
National Institute of Environmental Health Sciences

Research Triangle Park, NC 27709



Introduction

- ◆ 4-Methylimidazole (4-MI)



- ◆ 4-MI is used in the manufacture of pharmaceuticals, photographic and agricultural chemicals, dyes, and rubber
- ◆ 4-MI is a by-product of fermentation, found in food and tobacco smoke
- ◆ 4MI was nominated, together with 2-methylimidazole (2-MI), for study because of human exposure and lack of toxicity data
- ◆ 15-Day, 14-week (Tox. Report #67) and 2-year dosed feed studies conducted

14-Week Rat Study

At 0, 625, 1,250, 2,500, 5,000, 10,000 ppm

- Survival similar among male and female groups
- Body weights decrease was dose-related
- Food consumption reduced in dose-related manner
- Serum TSH, T4, T3 fluctuated with no patterns
- Tremors and ataxia observed in HD groups
- Anemia, hepatocytic vacuolization in HD groups
- Degenerative testes, atrophic prostate in HD males

Dose Levels Selected for 2-Year Rat Study

- ◆ Dose levels

0, 625, 1,250, or 2,500 ppm in male rats

0, 1,250, 2,500, or 5,000 ppm in female rats

- ◆ HD Levels Selected Based on Body Weights in 14-Wk Study

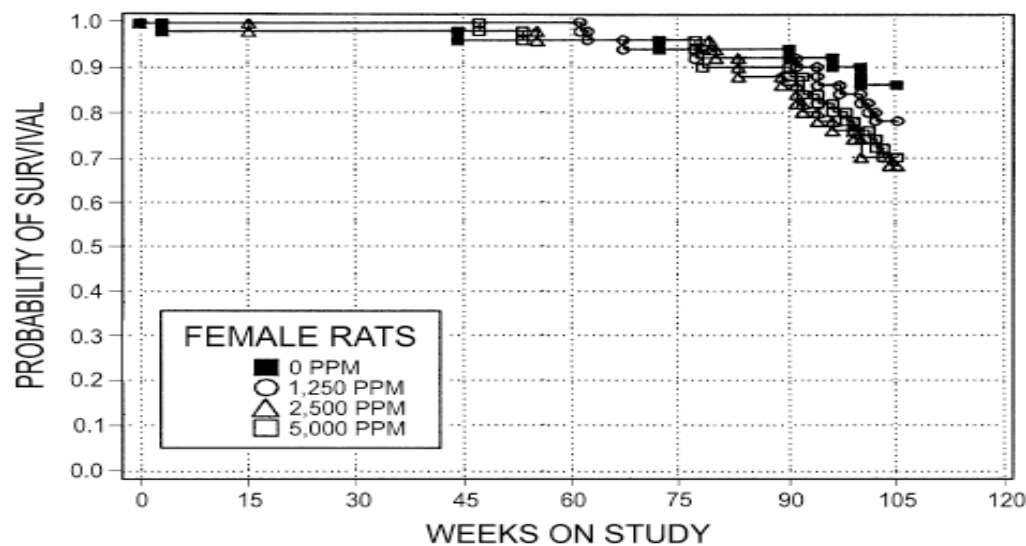
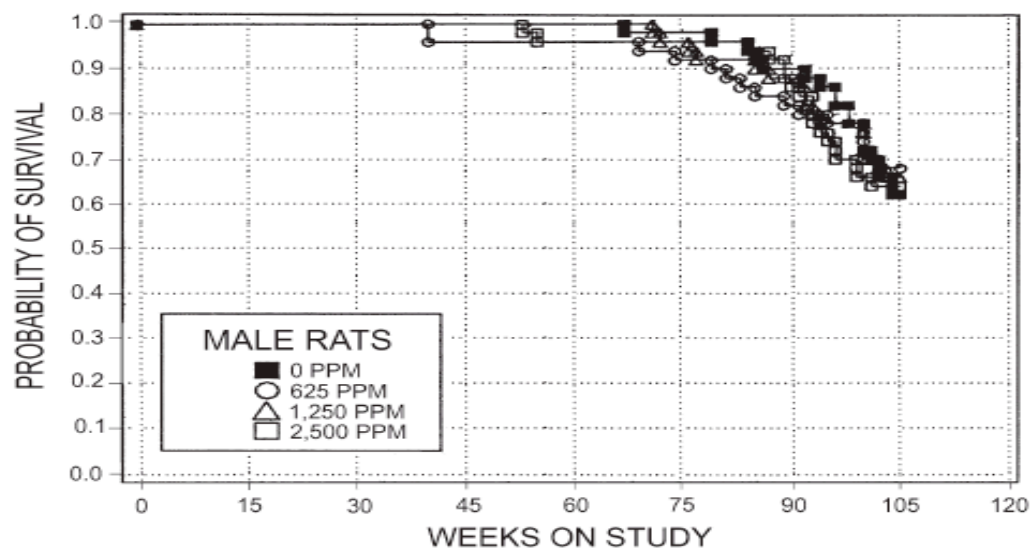
Body wt. of males at 2,500 ppm = 95% of control

Body wt. of females at 5,000 ppm = 94% of control

No treatment-related histopathologic changes

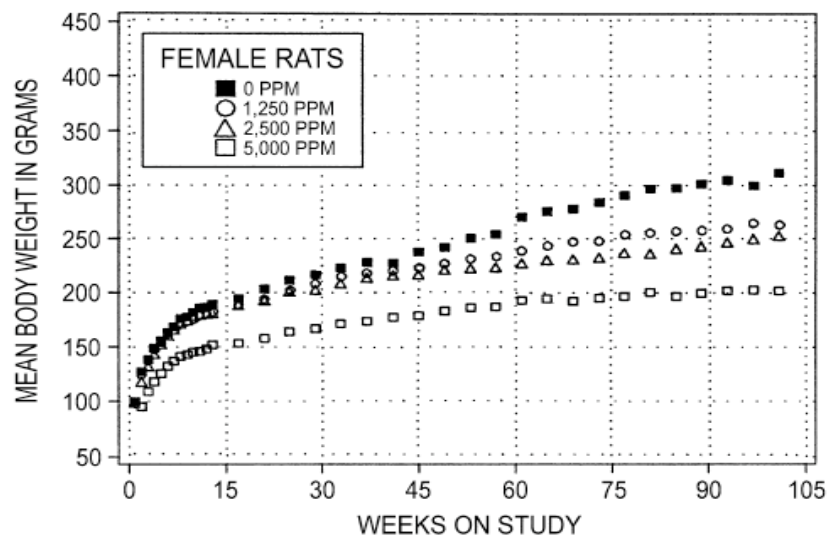
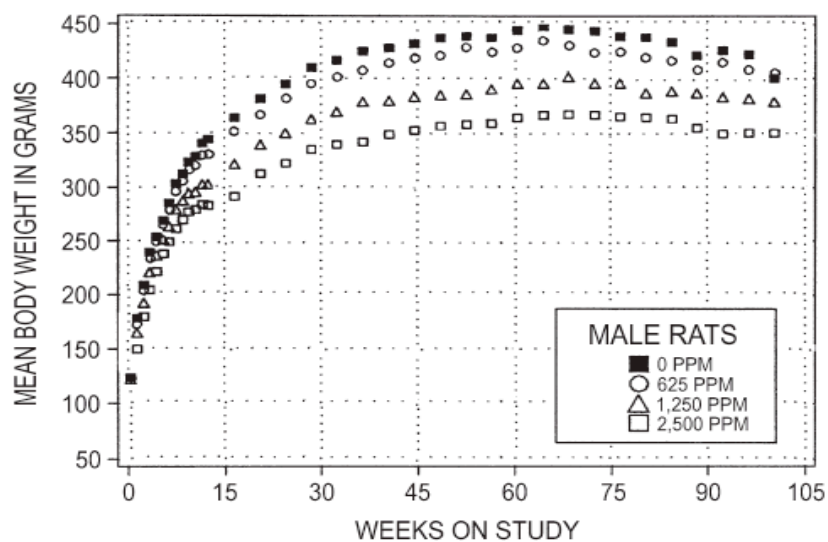


NTP
National Toxicology Program





NTP
National Toxicology Program



Neurobehavioral Findings in Female Rats

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Number Examined	50	50	50	50
Excitability	0	2	9	50
Hyperactivity	0	0	0	5
Impaired Gait	0	0	4	49
Clonic Seizure	0	0	21	36

-
- No histopathologic lesions in the brain, spinal cord, or sciatic nerve
 - No neurobehavioral findings observed in male rats

Incidences of Mononuclear Cell Leukemia in Rats

Male	<u>0 ppm</u>	<u>625 ppm</u>	<u>1,250 ppm</u>	<u>2,500 ppm</u>
	15/50 (30%)	18/50 (36%)	22/50 (44%)	20/50 (40%)
Female ^b	<u>0 ppm</u>	<u>1,250 ppm</u>	<u>2,500 ppm</u>	<u>5,000 ppm</u>
	9/50 (18%)	7/50 (14%)	16/50 (32%)	20/50* (40%)

^aHistorical incidence 121/510 (46.8%); range 30%-68%

^bHistorical incidence 246/510 (23.8%); range 12%-38%

*P<0.05,

Incidences of Nonneoplastic Hepatic Lesions in Male Rats

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
N	50	50	50	50
Histiocytosis	38(1.1) ^a	45* (1.4)	50**(1.9)	50**(2.3)
Inflammation	18(1.1)	32**(1.2)	31**(1.3)	36**(1.3)
Focal Fatty Change	21(1.5)	24 (1.8)	37**(1.9)	33**(2.5)
Eosinophilic Focus	4	3	7	12*
Mixed Cell Focus	5	7	11	27**

^a Average severity: 1=minimal, 2=mild, 3=moderate, 4=marked

* P < 0.05

**P < 0.01

Incidences of Nonneoplastic Hepatic Lesions in Female Rats

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
N	50	50	50	50
Histiocytosis	40(1.0) ^a	50 ^{**} (1.3)	48 ^{**} (1.9)	50 ^{**} (2.5)
Inflammation	17(1.2)	28 [*] (1.5)	34 ^{**} (1.8)	35 ^{**} (1.7)
Focal Fatty Change	16(1.2)	29 ^{**} (1.6)	29 ^{**} (2.0)	32 ^{**} (2.2)
Clear Cell Focus	20	32 ^{**}	23	27
Eosinophilic Focus	1	2	5	11 ^{**}
Mixed cell Focus	10	7	6	18 [*]

^a Severity: 1=minimal, 2=mild, 3=moderate, 4=marked

* P<0.05

**P<0.01

Increased Incidences of Nonneoplastic Lesions

Commonly observed lesions (of minimal to mild severity) that may have been exacerbated by 4-MI treatment:

- Males (1,250 and/or 2,500 ppm)
 - Prostate gland inflammation, chronic
 - Pituitary gland hypertrophy, focal
 - Thyroid gland follicle cyst
- Females (1,250, 2,500, and/or 5,000 ppm)
 - Thyroid gland follicle mineralization
 - Lung inflammation, chronic
 - Heart, cardiomyopathy
 - Pancreas, acinus, atrophy, focal

Decreased Incidences of Neoplasms in Rats

Male	<u>0 ppm</u>	<u>625 ppm</u>	<u>1,250 ppm</u>	<u>2,500 ppm</u>
Pituitary Adenoma	16/49	13/49	10/48*	7/48*
Adrenal Medulla Pheochromocytoma	10/50	6/50	3/50*	3/50*
Female	<u>0 ppm</u>	<u>1,250 ppm</u>	<u>2,500 ppm</u>	<u>5,000 ppm</u>
Pituitary Adenoma	29/48	19/50*	20/50	9/50**
Clitoral Gland Adenoma	8/50	1/50*	0/50**	0/50**
Mammary Fibroadenoma	24/50	6/50**	4/50**	1/50**
Uterus Stromal Polyp	16/50	5/50**	2/50**	2/50**

*P<0.05

**P<0.01

14-Week Mouse Study

At 0, 625, 1,250, 2,500, 5,000, 10,000 ppm

- Survival similar among male groups and female groups
- Body weights decreased in dose-related manner in males and females
- Serum T4, T3 levels fluctuated with no specific patterns
- No treatment related histopathologic changes

Dose Levels Selected for 2-Year Mouse Study

- ◆ Dose Levels

Males : 0, 312, 625, 1,250 ppm

Females: 0, 312, 625, 1,250 ppm

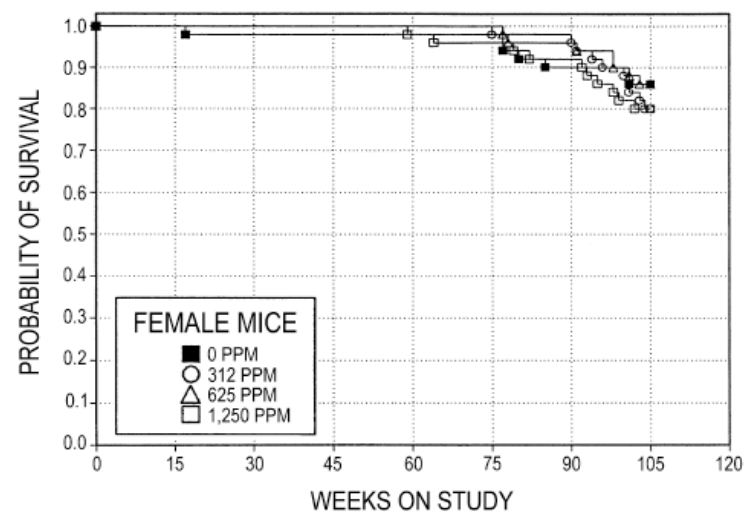
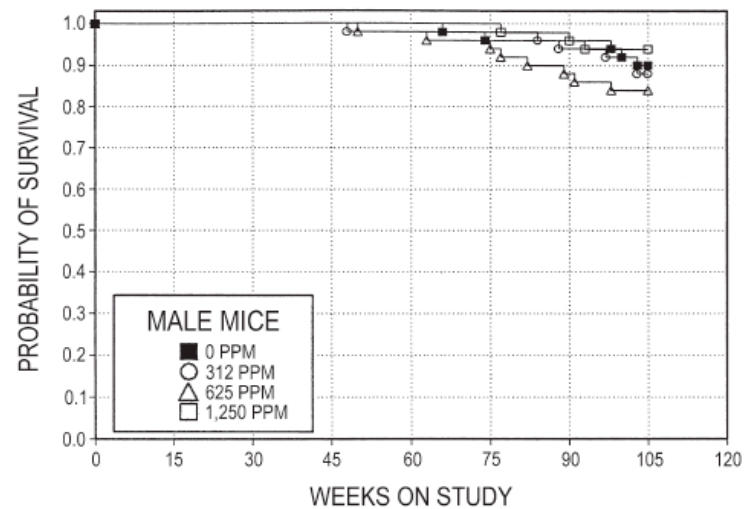
- ◆ HD Levels Selected Based on Body Weights in 14-Wk Study

Males at 1,250 ppm = 93% of controls

Females at 1,250 ppm = 88% of controls

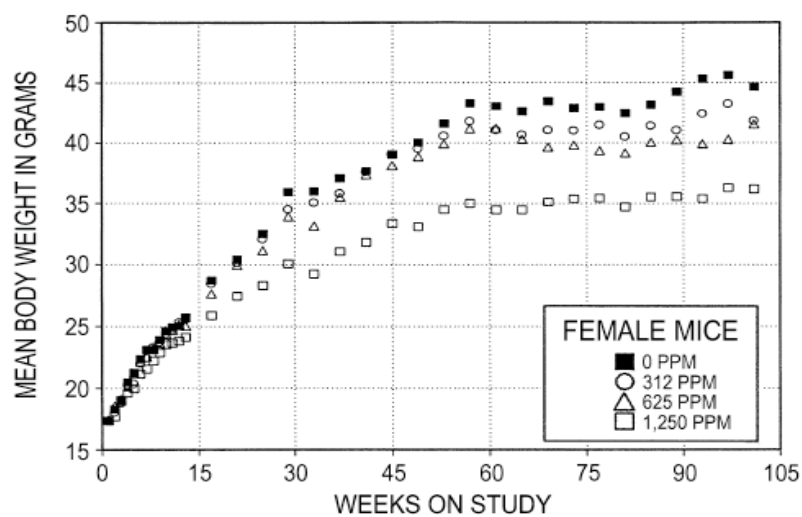
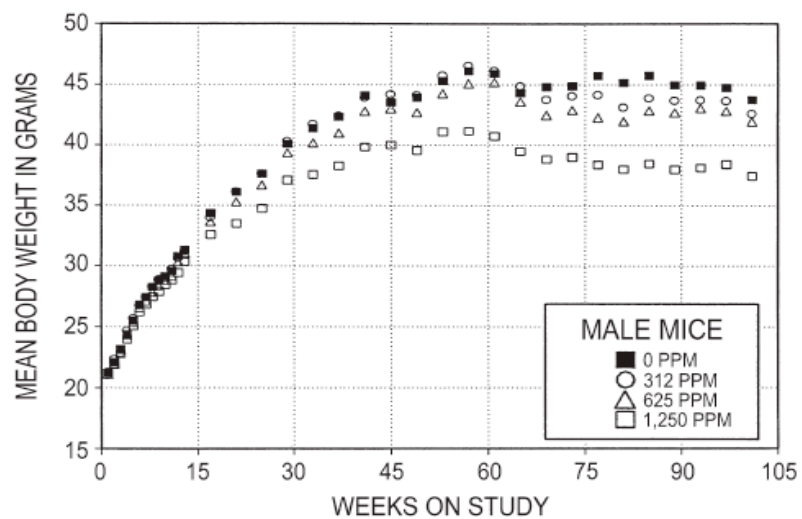


NTP
National Toxicology Program





NTP
National Toxicology Program



Incidences of Lung Lesions in Mice

	0 ppm	312 ppm	625 ppm	1,250 ppm
N	50	50	50	50
Male				
Alveolar Epithelium Hyperplasia Alveolar/bronchiolar	7(2.0) ^a	3 (1.0)	1(2.0)	9(1.9)
Adenoma	8	11	13	15
Carcinoma	2	4	4	8*
Ad or Ca (combined)	9	13	16	22**
Female				
Alveolar Epithelium Hyperplasia Alveolar/bronchiolar	3(1.7)	2 (2.5)	3(1.7)	11*(1.9)
Adenoma	0	8**	16**	8**
Carcinoma	3	0	2	7
Ad or Ca (combined)	3	8	17**	14**

^a Average severity: 1=minimal, 2=mild, 3=moderate, 4=marked

* P < 0.05

**P < 0.01

Genetic Toxicology

- 4-MI was not mutagenic in Salmonella tests with or without S9
- 4-MI did not induce micronuclei in bone marrow cells or peripheral red blood cells

Toxicokinetic Studies of 4-MI in Rats and Mice

Following single gavage dose of 10, 50, or 100 mg/kg data showed:

- First-order absorption and elimination kinetics
- Absorption and elimination kinetics were dose-dependent
- Elimination half-lives increased with dose implying saturation of metabolism or excretion.
- A PBPK model based on these kinetic data predicts increasing concentration of 4MI during the 2-year study in male rats exposed to 2,500 ppm and female rats at all three exposures. Plasma concentrations from animals at those exposures did not show an increase with time, implying no saturation with dosed feed exposure.

Conclusions

- **No evidence** of carcinogenic activity of 4-MI in male rats at 625, 1,250, or 2,500 ppm
- **Equivocal evidence** of carcinogenic activity of 4-MI in female rats based on increased incidences of mononuclear cell leukemia
- **Clear evidence** of carcinogenic activity of 4-MI in male and female mice based on increased incidences of alveolar/bronchiolar neoplasms



NTP
National Toxicology Program

NTP Technical Reports Review Subcommittee Meeting

4-Methylimidazole TR 535

